Synopsis of Ph.D. Research Work Entitled

Study of the Ameliorating Effect of *Cissus quadrangularis* (Lin.) on freshwater fish *Heteropneustes fossilis* (Bloch) under Metal Stress

To be undertaken by

Kumari Babli

Department of Zoology,

Ranchi University, Ranchi

Under Supervision of

Dr. Veenapani

Department of Zoology,

Nirmala College

Ranchi University, Ranchi

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SATE OF ART

Solubility of Aluminium is a direct function of ambient pH, being at a minimum at around pH 5.5 and increasing towards both extremes of pH scale. Aluminium mobilization in its soluble form from soil to aquatic ecosystems is an important consequence of acidification. Fish kills resulting from the uncontrolled release of acid and metals from mine wastes into receiving streams have been reported from world wide areas in which hard rock mining, milling and smelting activities have occurred (Walker et al., 2001; Cronan and Schofield, 1979). The biochemical parameters are used as an index of fish physiological status. Metal toxicity is responsible for physiological stress in the test species (Naskar *et al.*2004, Bhattacharjee, 2008).

On a molar basis, aluminium is not very toxic in comparison with many other metals, but is a serious problem in acidified areas. Aluminium toxicity hypothesis lies in the fact that, Al in soils is mobilized by acidic deposition. At pH below 5.0 aluminium may become the dominant buffering effect in the soil by removing the hydrogen ions. The reaction for most commonly occurring aluminium hydroxide of soil, gibbsite is as follows:

$$Al(OH)_3 + 3H+ \text{ ----- } Al^{3+} + 3H_2O$$

Besides providing the buffering capacity by removal of hydrogen ions, there is also the resulting liberation of highly toxic Al^{3+} ions that enter solution with runoff into streams and lakes.

Abnormalities have been found in SEM studies of gills of fish exposed to sublethal concentration of aluminium. These are epithelial hypertrophy and hyperplasia as well as partial to complete lamellar fusion (Veenapani et al,2013). Under toxic condition survival rate of fishes decreases.

Cissus quadrangularis L. is known as veldt grape or devil's backbone . It is named as Hadjod in hindi, Vajravalli in Sanskrit. *Cissus quadrangularis* L. is a perennial plant belongs to the family vitaceae and is an indigenous medicinal plant of India. It is full of antioxidants and vitamins that has been used and is recorded in ancient ayurvedic texts. It gives pain relief and anti inflammatory effect at the level of aspirin or ibuprofen without any side effect associated with the drugs (Panthong *et al*., 2007). *Cissus*, the plant is also rich in antioxidants, vitamin C and beta-carotene. As analyzed, *Cissus quadrangularis*(CQ) *c*ontain ascorbic acid 479 mg. and carotene 267 units per 100g of freshly prepared paste in addition to calcium oxalate (Chidambara Murthy *et al*.,2003). The antioxidant effects of CQ extract on the immune system of fish under normal and stress conditions are largely unknown. Therefore, the present study aim to investigate the ameliorating effect of *Cissus quadrangularis* (Lin.) on freshwater fish *Heteropneustes fossilis* (Bloch) under acid induced aluminium metal stress. The antioxidant properties of natural substances is believed to be a promising way to prevent and cure diseases. The assessment of the immunomodulatory

activity and antioxidant properties of plant extracts is an expanding area of research. Furthermore, medicinal plants used for immunomodulation can provide potential alternatives to conventional chemotherapies for a variety of diseases, especially when the host defense mechanism has to be activated under the conditions of impaired immune response.

The morphology of the immune system is quite different between fish and mammals. Most obvious is the fact that fish lack bone marrow and lymph nodes. Instead, the head kidney serves as a major lymphoid organ, in addition to the thymus and spleen (Press & Evensen, 1999). Environmental quality influences the fish's physiological well-being. Overall physiological status of the fish host is determined by the husbandry practice, environmental quality, the fish's nutritional well-being and the pathogen; fish stressed by one of these factors are more susceptible to infection (Plumb & Hanson, 2011). Effects of herbal remedies for the fishes have been beneficial such as reducing stress response, increasing the activity of innate parameters and improving disease resistance (Magnadóttir, 2010). Antioxidant property of *Cissus quadrangularis* L. has been investigated in many recent studies (Murthy, et al,2003; M.Jainu,2004,2005; M.Prabhavathi,2016).

PROBLEM TO BE INVESTIGATED:

Research envisages development of a simulated system in which the test species will be treated with sublethal dose of metal aluminium in acidified ambient water by an acute toxicity test. Damage will be assessed in terms of certain biological indicator of stress. The recovery of the damages recorded will be assessed with the treatment of same fishes with ameliorating agent *Cissus quadrangularis*(Lin.) methanolic stem extract. Effect of *Cissus quadangularis*(Lin.) methanolic stem extract will also be evaluated under normal condition. In the present study the following activities will be taken up in freshwater fish *Heteropneustes fossilis (bloch)* with metal toxicity:

1. Measurement of inflammatory mediators (Oxidative stress biomarkers).
2. SEM of gill tissues and erythrocytes for anti-inflammatory effect of *Cissus quadrangularis*(Lin.).
3. Histological examination to access the changes in the gills and liver tissues.
4. Study of biochemical parameters like glucose content, total cholesterol content, protein content, acid phosphatase & alkaline phosphatase.
5. Measurement of Haematological parameters.
6. Antioxidants markers analysis in tissue homogenates

OBJECTIVE OF THE INVESTIGATION:

The objective of the present work is to access the anti-inflammatory, antioxidant properties of *Cissus quadrangularis* (Lin.) on freshwater fish *Heteropneustes fossilis (bloch)* . Study will be carried out under normal condition and also with acid induced aluminium toxicity to investigate the ameliorating effect of methanolic extract of *Cissus quadrangularis*(Lin.).

Parameters to be measured will be Reduced Glutathione (GSH) for anti-inflammatory response. For antioxidants analysis modulations in the activity of Superoxide dismutase (SOD), Catalase (CAT) and Lipid peroxidation (LPO) in tissue homogenates will be measured. Biochemical, Haematological and SEM and Histopathological studies will also be done to measure the stress level and recovery of fish.

HYPOTHESIS: Aluminium has been recognized as the principal toxicant posing a serious threat to aquatic organisms including fish kills at low pH in soft waters. *Cissus quadrangularis* L. has anti-inflammatory and antioxidant properties. The study includes the evaluation of possible ameliorating effect of *Cissus quadrangularis* L. in recovery under metal stress condition in fresh water fish *Heteropneustes fossilis* (Bloch).

REVIEW OF STATUS OF RESEARCH AND DEVELOPMENT IN THE SUBJECT

INTERNATIONAL STATUS

1. **A. Panthong, et al (2007)** Methanol extract of *C. quadrangularis* (CQ) were assessed for analgesic and anti-inflammatory activities and venotonic effect in mice. The results revealed positive impact of *C. quadrangularis* for the treatment of pain and inflammation associated with hemorrhoid as well as reducing the size of hemorrhoids.

NATIONAL STATUS

1. **K.N. Chidambara Murthy, et al (2003)** Extracts of *Cissus quadrangularis* L. were tested for antioxidant activity by β-carotene linoleic acid model and also by 1,1-diphenyl-2-picrylhydrazyl model. The ethyl acetate fraction of both fresh and dry stem extracts at a concentration of 100 ppm showed 64.8% antioxidant activity in the β-carotene linoleic acid system and 61.6% in the 1,1-diphenyl-2-picrylhydrazyl system. This fraction showed the presence of sterols, vitamin C, and tannins as phytoconstituents. Results revealed *C. quadrangularis* has antioxidant property.
2. **M. Jainu and C.S. Shyamala (2004)** The effects of *Cissus quadrangularis* extract on aspirin-induced ulceration in rats with respect to its antioxidant status, lipid peroxidation, quantity of mucus and alkaline phosphatase and myeloperoxidase (MPO) activities was estimated and it was found that *Cissus quadrangularis* extract protects against aspirin-induced gastric mucosal lesions in rats possibly through its antioxidative action.
3. **M. Jainu, CS. Devi (2005)** The present study was performed to evaluate the effect of the methanolic extract of Cissus quadrangularis (CQE) against free radical damage. The results suggest that CQE showed inhibition of lipid peroxidation, free radical production and increase in antioxidant enzymes activities, which reveal its antioxidant property and responsible for the therapeutic action against tissue damage.
4. **M. Jainu, CSS. Devi, Vijaimohan K.(2005)** This study evaluated the ethanol extract of *Cissus quadrangularis* (CQE) against the gastric toxicity induced by aspirin in rats. Findings suggest that the gastroprotective activity of CQE could be mediated possibly through its antioxidant effect as well as by the attenuation of the oxidative mechanism and neutrophil infiltration.
5. **M. Jainu and Kunju V.M.(2008)** The study was investigated to see the effect of ascorbic acid isolated from Cissus quadrangularis extract on inflammatory cytokines and growth factors in non-steroidal anti-inflammatory drug (NSAID) induced gastric ulcer.The findings suggest that CAA prevents gastric ulcer formation due to its immunomodulatory effect, antioxidant activity along with the ability to modulate prostaglandin synthesis and up-regulation of the growth factors.
6. **Garima Mishra, et al(2010)** This review highlights some of the phytochemical and pharmacological aspects of *Cissus quadrangularis,* has been reported to posses bone fracture healing, antibacterial, antifungal,antioxidant, anthelmintic, antihemorrhoidal and

analgesic activities. *Cissus quadrangularis* Linn. has been recognized as a rich source of carotenoids, triterpenoids and ascorbic acid and is proved to have potential for medical effects,

7. **Priyanka Vijay and Rekha Vijayvergia (2010)** This study evaluated the analgesic anti-inflammatory and antipyretic activity of ethanolic extract of Cissus quadrangularis in albino rats. Ethanolic extract significantly reduced the edema induced by carrageenan On the analgesic property writhing was significantly reduced. Its effects on antipyretic activity as it significantly reduces fever .
8. **Priyanka Yadav, et al.(2014)** Phytochemical and phytoanalytical tests demonstrated that *C. quadrangularis* is rich in polyphenolic and flavonoid content. *C. quadrangularis* also showed promising immunomodulatory potential on various models like DTH, carbon clearance test and effect on serum immunoglobulins on rats.
9. **P.S. Radhakrishnan, et al (2014).***Cissus quadrangularis* was used to assess the growth promoting ability, enhancement of muscle biochemical constituents and profiles of amino acid in the freshwater prawn, *Macrobrachium malcolmsonii*. The survival of prawn significantly improved in herbal incorporated feed. In addition, the growth parameters, such as weight gain, specific growth rate and protein efficiency rate were significantly higher in herbal diets fed groups when compared with control. The activities of digestive enzyme, biochemical constituents, profile of amino acid, energy utilization parameters, concentrations of vitamin C and E were significantly elevated.
10. **Aruldoss K. et al.(2014)** This study investigated the effect of *Cissus quadrangularis* on glycogen content in fresh water fish, *Oreochromis mossambicus* and found that *Cissus quadrangularis* is beneficial to the growth and development of fishes.
11. **Prabhavathi R. M., Prasad M. P.and Jayaramu M**.(**2016**) In the present investigation antioxidant property of Cissus quadrangularis Linn was determined by DPPH assay, DMPD assay and reducing power assay The methanolic extracts showed a better antioxidant property for all the assays when compared to the ethanolic and petroleum extract of the samples.

SIGNIFICANCE OF THE STUDY AND ITS RELEVANCE IN THE CONTEXT OF CURRENT STATUS

Plant extracts and products provide natural remedies for many disorders. Phytotherapy has come as a handy and viable alternative to chemotherapy. Herbal medicines are effective, economic, eco-friendly and farmer friendly, easily biodegradable, non-habit forming, non-resistance forming, non-narcotic, and without much side effects. Besides, the raw materials are inexpensive, renewable, locally available, user friendly, and can be easily prepared (R. Harikrishnan and C. Balasundaram,2005) The application of Phytotherapy in field of fish and fisheries is a relatively new area of research. The herbs may be used in many ways such as garden fresh, dried, powdered, juices, or extracted (in various solvents like water, alcohol, acetone, ether, etc), or essential oils. The bioactive natural products are important source of drug leads, but most of the times their modes of action are unknown. Thus, elucidation of their physiological targets is essential for understanding their therapeutic effects (J.Won, 2005).

In and around Jharkhand, 90% of the soil is red, laterite and acidic soil type. Presence of excess of aluminium ores (bauxite) of about 61.1 million tons open up the possibilities of mobilization of aluminium from soil to nearby water bodies. This may result into reduction of fish fauna as well as economic loss as fish food.

Very little work has been done on the biological activity and plausible medicinal application of plants in combating the numerous diseases and stressful situations facing the

aquaculture industry. More serious investigations are required to harness the therapeutic properties of medicinal plants to combat problems in aquaculture (R. P. Raman,2017). The fisheries fraternity will be benefitted from the work carried out on phytotherapy of fishes and will be helpful in combating the problems of fisheries world.

RESEARCH DESIGN AND METHODOLOGY:

It undertakes the following aspects for a detailed study:

I. To design a simulated system of ambient water pH less than 5.6 and expose the test fishes in replicates of 20 individuals each for a period of 7 days at one fifth dose of LC50 dose of aluminium to induce physiological stress. Another group of fishes will be treated only with methanolic extract of stem of *Cissus quadrangularis*(Lin.) for 7 days. A control set with same number of fishes will also be maintained alongside.

II. To treat the fishes under acute phase of toxicity with $1/5^{th}$ dose of LC50 and also treat same lot of fishes with methanolic extract of *cissus quadrangularis* to ascertain its ameliorating effect in aluminium toxicity.

III. To prepare SEM preparations of gills and erythrocytes of control, acid metal treated , ameliorating agent-treated individuals to assess the extent of damage as well as level of recovery.

IV. Histopathological studies will be done for gills and liver tissues.

V. Serum glucose level, serum protein , serum cholesterol and other biochemical parameters will be studied to measure change in stress level.

VI. Haematological parameters will also be studied for immune response and recovery.

VII. Measurement of inflammatory mediators (Oxidative stress markers) will be by Reduced Glutathione (GSH).

VIII. The impact of such acute toxicity and recovery tests shall be interpreted in terms of modulations in the activity of Superoxide dismutase (SOD), Catalase (CAT) and Lipid peroxidation (LPO) in tissue homogenates for antioxidants analysis.

Tools to be used in the collection of data:

1. Aquaria of Required dimension
2. Soxelet
3. Spectrophotometer
4. Scanning Electron Microscope
5. Tissue Homogenizer

Year wise work plan

1st Year:

Literature survey

Design of experimental setup
Preparation of botanical extract
SEM preparations of gills & erythrocytes

2nd Year:
Histopathological study in tissue of gills and liver
Measurement of Biochemical parameters

3rd Year:
Study of Haematological parameters
Measurement of Antioxidant property of *Cissus quadrangularis* (Lin.)

4th Year:
Statistical Analysis and Data compilation
Thesis writing and submission

5th Year:
Final Viva-voce

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